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CRDEC-TR-217

THE EFFECTS OF GRAPHITE FLAKES IN SOIL ON TERRESTRIAL PLANTS

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CHEMICAL COMMAND

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13. ABSTRACT (Maximum 200 words)

Graphite flakes were tested for their toxicity to corn and cucumber. The flakes were tested at 0 (control), 0.05, 0.10, and 0.50% concentration by weight. No lethal or sublethal effects on corn plants were produced. The two-way analysis of variance (ANOVA) for plant heights showed no significant ($p < 0.05$) difference between control plants and those grown at other concentrations. The ANOVA showed no significant ($p < 0.05$) difference in the mean dry weights of corn plants. The ANOVA of mean plant heights for cucumbers indicated a significant ($p < 0.01$) difference. The Newman-Keuls Test showed a significant ($p < 0.01$) difference between the 0 and 0.05% and the 0.10 and 0.50% concentrations. However, the ANOVA of the mean dry weights of cucumber plants revealed no significant ($p < 0.05$) difference. This study indicates that graphite flakes significantly increases heights of cucumber plants at the 0.10 and 0.50% levels. A second cucumber study was conducted to confirm this observation. The ANOVA for this study indicated that graphite flakes had no significant ($p < 0.05$) difference in either the mean plant heights or the mean dry weights. It is concluded that graphite flakes should have no adverse environmental effect if field-release levels correspond to those used in this study.

14. SUBJECT TERMS

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PREFACE

The work described in this report was authorized under Project No. 89AH01011, XM-55 Project. This work was started in July 1988 and completed in July 1989.

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This report has been approved for release to the public.

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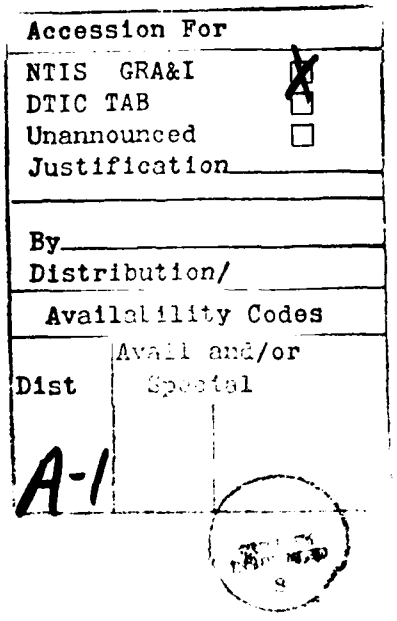

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THE EFFECTS OF GRAPHITE FLAKES IN SOIL ON TERRESTRIAL PLANTS

1. INTRODUCTION

The purpose of this study was to develop baseline environmental phytotoxicity data on graphite flakes. Phytotoxicity testing measures plant growth using plant height and biomass. The method is relatively rapid, simple and inexpensive. Graphite flakes are components of smoke and obscurant systems used by the military. The determination of environmental toxicity data on graphite flakes is important because the information supports environmental documentation requirements and ensures that unforeseen environmental problems are not created.

The study of the toxic effects of chemicals on plants is important for several reasons. One reason is that chemicals may adversely damage the ecosystem and negatively impact wildlife. In addition, chemicals may enter human food chains through processes associated with soil/plant interactions, uptake, translocation, and accumulation in food and forage crops. The type of phytotoxicity testing we used employs plant height and dry weight measurements as plant growth indicators. This project developed baseline data on the effects of graphite flakes on monocot (corn) and dicot (cucumber) plants.

2. METHODS AND MATERIALS

The test methods used for phytotoxicity studies were adapted from the U.S. Environmental Protection Agency's (USEPA) Environmental Effects Test Guidelines (Early Seedling Growth Toxicity Test).* The plant species selected for use in this study, chosen from the USEPA's list of recommended crops, were corn (Zea mays L., cv. Silver Queen) and cucumbers (Cucumis sativus L., cv. Straight Eight). Corn and cucumber seeds were obtained from the Meyer Seed Company (Baltimore, MD) and sorted to ensure uniform size. Damaged and malformed seeds were discarded.

The soil used for these tests was obtained from a site near Winters Run Creek (Edgewood, MD). The soil was an acidic Joppa gravelly sandy loam [loamy-skeletal, siliceous, mesic typic Hapludult], having a moderate cation exchange capacity, organic matter content, and nutrient status. The sandy loam-textured soil was air-dried and seived to pass a 2-mm screen. The respective physical and chemical properties of the soil are given in Table 1.

The graphite flakes (CAS # 7782-42-5) were purchased from The Asbury Graphite Mills, Incorporated (Asbury, NJ). The physical and chemical characteristics of graphite flakes are given in Table 2.

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*Environmental Effects Test Guidelines, EPA 560/6-82-002, U.S. Environmental Protection Agency, Washington, DC, 1982.

Table 1. Physical and Chemical Characteristics of Winters Run Soil

Soil Parameters*	
Mechanical Analysis:	**Soil Analysis:**
% sand - 61	NO_3 (Lb/A) - 19
	P_2O_5 (Lb/A) - 30
% silt - 29	K_2O (Lb/A) - 50
	Ca (Lb/A) - 1300
% clay - 10	Mg (Lb/A) - 280
	Mn (Lb/A) - 26
% organic matter - 4.8	Zn (Lb/A) - 17.3
	Cu (Lb/A) - 4.6
Texture - sandy loam	CEC (meq/100 g) - 7.9
	pH - 4.9

*Determined by the Soil Testing Laboratory, University of Maryland, College Park, MD

Table 2. Chemical and Physical Characteristics of Graphite Flakes

Characteristics (% by weight)	Minimum	Maximum
Carbon	96.6	----
Moisture and volatiles	----	0.10
Ash	----	3.30
Silica (in ash)	----	2.00
Particle size: retained on a No. 325 sieve	----	0.10

The flakes were mixed with soil on a weight basis, using a Hobart food blender. The same procedures were followed for both corn and cucumbers. The concentrations of graphite flakes used in each independent study were 0 (control), 0.05, 0.10, and 0.50% by weight. In each experiment, individual treatment pots were prepared in triplicate for each concentration used. Treatment pots were prepared by placing a 10 cm^2 piece of cheesecloth in the bottom of each 10 cm flower pot, followed by 100 g of pea gravel (3 to 5 mm diameter). A second piece of cheesecloth was placed on top of the pea gravel. The spiked soil was then added to the pots. Fourteen seeds were planted to a depth of 5 to 6 mm in each pot. Pots were watered on a weight basis to provide a field-capacity moisture level of 22%. The experimental design for the graphite flakes

studies on both corn and cucumber plants was a complete randomized block design with blocks of treatments replicated in triplicate (Table 3).

Table 3. Experimental Design for Corn and Cucumber Plants

Test Concentration	Flower Pot No.	Graphite Flakes: Soil (g)
Control (0%)	1, 2, 3*	0:325
0.05%	4, 5, 6	0.1625:324.8375
0.10%	7, 8, 9	0.325:324.675
0.50%	10, 11, 12	1.625:323.375

*Replicates in rows (e.g., 1, 2, 3); blocks in columns (e.g., 1, 4, 7, 10)

Individual treatment pots within blocks were randomized once a week for the 2-week growth period. The pots within each block were rotated three times each week because of the phototropic response to the sun. The plants were grown in a greenhouse from July through September.

Following germination, after 50% of the seedlings had emerged, the plants were thinned to the 10 most uniform plants in each pot and grown for 14 days following emergence (day 1). Plant heights were measured in situ twice per week following thinning, and a final measurement was taken at harvest on day 14. Plant heights were measured (nearest 0.1 cm) from the soil level to the plant apex. Plants were evaluated each day for chlorotic/necrotic plant tissue, stunting/ enhancement of growth, structural abnormalities, unusual color, change in the shape of the leaves, etc.

On day 14, after the final height measurement was made, the plants were cut 1 mm above the soil, and the 10 plants from each pot were placed into a paper bag. The bags were put into a drying oven for three days at 65 °C; then, the plants were reweighed to obtain the dry weights.

The methods used to statistically analyze the data were the two-way Analysis of Variance (ANOVA) and the Newman-Keuls pairwise comparison of means.*

*Tallarida, R.J., and Murray, R.B., Pharmacological Calculation System Based on "Manual of Pharmacologic Calculations with Computer Programs," 2nd ed., Springer-Verlag, New York, NY, 1986.

3. RESULTS AND DISCUSSION

3.1 Effects of Graphite Flakes on Corn Plants.

The plant heights of corn grown in soil amended with graphite flakes were taken at harvest, 14 days after emergence (Appendix A, Table A-1). The mean plant heights for corn are given in Figure 1. Treatment means for corn plant heights ranged from a low of 346.8 $\pm$ 69.2 mm for the control plants (0% concentration) to a high of 373.9 $\pm$ 59.8 mm for the 0.05% level. The ANOVA of plant heights indicated no significant ($p < 0.05$) difference between treatments with no significant ($p < 0.05$) difference between blocks (Appendix B, Table B-1).

The dry weights of corn plants (Appendix C, Table C-1) were also used as a plant growth indicator. The ANOVA of the dry weights of corn plants grown in soil amended with graphite flakes indicated that there was no significant ($p < 0.05$) difference.

3.2 Effects of Graphite Flakes on Cucumber Plants.

The heights of cucumbers grown in soil amended with graphite flakes were taken at harvest, 14 days after emergence (Appendix A, Table A-2). The mean plant heights are given in Figure 2. Treatment means for cucumber plant heights ranged from a low of 100.8 $\pm$ 12.5 mm for the control plants (0% concentration) to a high of 117.5 $\pm$ 14.9 mm for the 0.50% level. The ANOVA (Appendix B, Table B-2) of the heights of cucumber plants treated with graphite flakes indicated a significant ($p < 0.01$) difference between treatments with no significant ($p < 0.05$) difference between blocks. A Newman-Keuls Test (Appendix D, Table D-1) was performed to determine whether differences in plant heights were due to treatment or other factors. This test showed that there was no significant ($p < 0.05$) difference between either the control and the lowest concentration (0.05%) or the 0.10 and 0.50% levels. However, there was a significant ($p < 0.01$) difference between the two lower concentrations (0 and 0.05%) and the two higher concentrations (0.10 and 0.50%). When the dry weights (Appendix C, Table C-2) of these cucumber plants were statistically analyzed, the ANOVA showed that there was no significant ($p < 0.05$) difference in dry weights.

The effects of graphite flakes on cucumber plants indicated a significant ($p < 0.01$) difference between treatments for plants heights. To confirm this observation, a second study using the same concentrations (0, 0.05, 0.10 and 0.50%) was conducted for cucumber plants only . The heights of cucumber plants (Appendix A, Table A-3) in the second study were also taken at harvest, 14 days after emergence. The mean plant heights for these cucumber plants are given in Figure 3. The ANOVA (Appendix B, Table B-3) of the plant heights of cucumbers treated with graphite flakes indicated no significant ($p < 0.05$) difference between either treatments or blocks. The dry weights of cucumber plants are given in Appendix C, Table C-3. The ANOVA indicated no significant ($p < 0.05$) difference in the dry weights of cucumber plants grown in soil amended with graphite flakes in the second study.

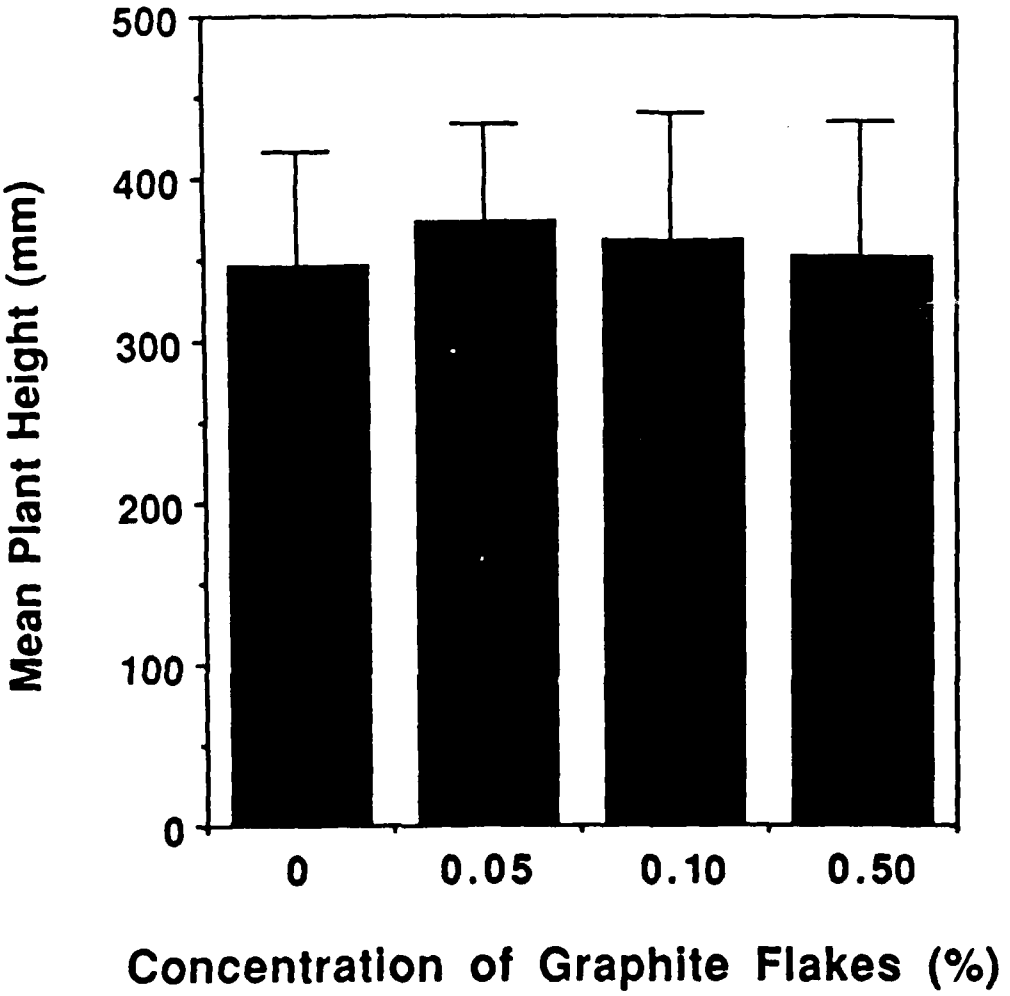

Figure 1. Effects of Graphite Flakes in Soil on the Mean Plant Height of Corn

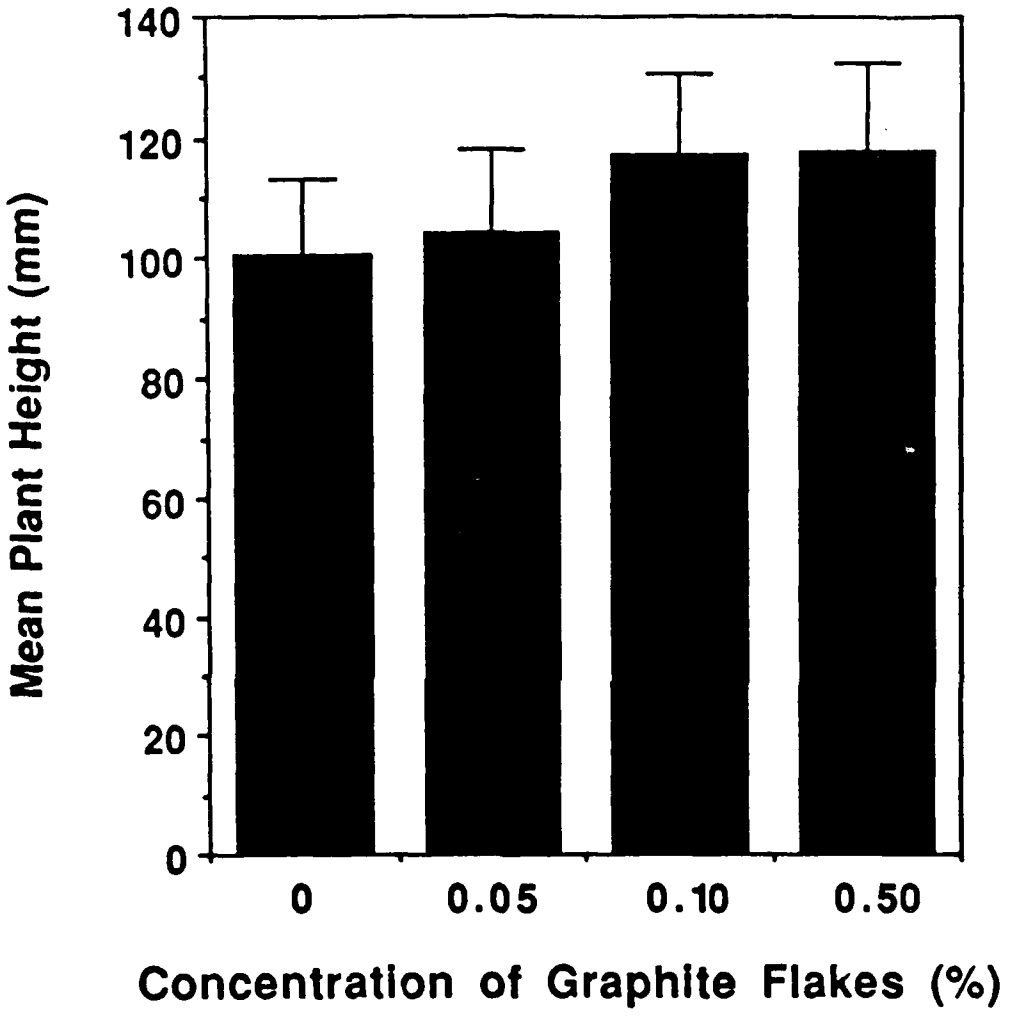

Figure 2. Effects of Graphite Flakes in Soil on the Mean Plant Height of Cucumber

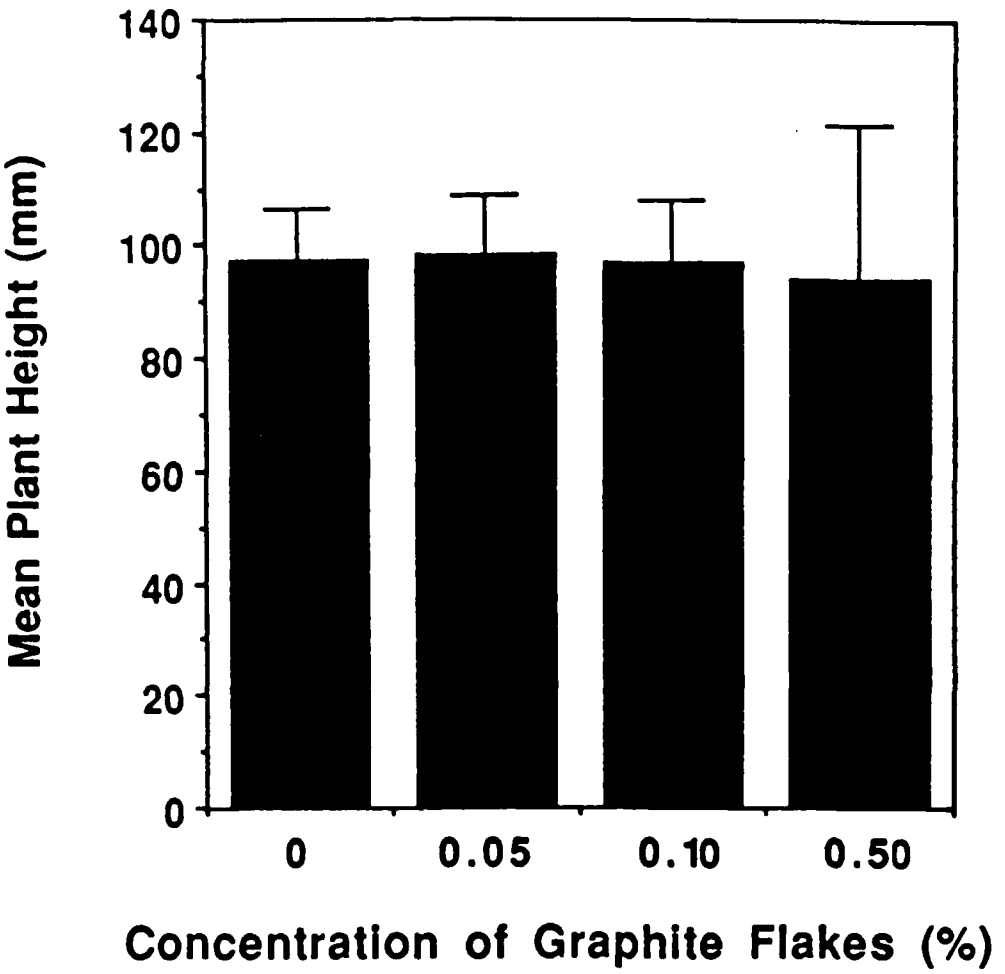

Figure 3. Effects of Graphite Flakes in Soil on the Mean Plant Height of Cucumber (Second Study)

4. CONCLUSIONS

Graphite flakes produced no lethal or sublethal effects on corn or cucumber plants at concentrations used for this study (through the 0.50% level). These results indicate that graphite flakes should not have an adverse effect on terrestrial plants if field-release levels approximate those used in this study.

The plant height data from the first study of cucumbers grown in soil amended with graphite flakes indicates that graphite flakes enhanced only the growth in height of cucumber plants at the 0.10 and 0.50% concentrations because there was no significant ($p < 0.05$) difference in dry weights. This enhancement may result from the graphite flakes permitting the young cucumber roots to penetrate the soil better. Another aspect is that the graphite flakes may have increased either the aeration or the water holding capacity of the soil, thus allowing the cucumber plants to absorb more oxygen and nutrients from the soil.

The results of the studies on both corn and cucumber plants indicate that graphite flakes do not exhibit lethal or sublethal effects (based on plant heights and dry weights) when subjected to concentrations up through the 0.50% level. Therefore, graphite flakes should not have an adverse effect on the environment when field-release concentrations are at or near 0.50% by weight in soil.

APPENDIX A
PLANT DATA
HEIGHTS OF CORN AND CUCUMBER PLANTS GROWN
IN SOIL AMENDED WITH GRAPHITE FLAKES

Table A-1. Heights (mm) of Corn Plants on Day 14, Grown In Soil Amended With Graphite Flakes.

Replicate No.	Plant No.	Plant Heights (mm)			
		0%	0.05%	0.10%	0.50%
I	1	315	369	397	322
	2	301	407	259	244
	3	416	255	296	234
	4	366	372	260	252
	5	321	370	397	260
	6	295	237	427	342
	7	294	450	418	285
	8	460	364	425	413
	9	458	474	369	471
	10	269	375	257	344
II	1	306	364	419	305
	2	431	378	256	491
	3	419	324	350	365
	4	298	295	345	410
	5	425	370	340	407
	6	310	422	360	453
	7	278	389	341	435
	8	380	454	272	383
	9	400	345	396	397
	10	258	371	259	358
III	1	334	448	458	234
	2	342	374	394	218
	3	332	284	401	223
	4	228	297	347	241
	5	216	418	275	481
	6	367	400	482	382
	7	415	487	480	381
	8	432	386	429	439
	9	431	377	231	397
	10	306	360	510	377
Mean:		346.8	373.9	361.7	351.5
Std. Dev.:		69.2	59.8	78.2	84.1

Table A-2. Heights (mm) of Cucumber Plants on Day 14, Grown In Soil Amended With Graphite Flakes.

Replicate No.	Plant No.	Plant Heights (mm)			
		0%	0.05%	0.10%	0.50%
I	1	96	97	126	108
	2	96	93	116	100
	3	104	96	130	126
	4	116	102	118	113
	5	108	100	125	120
	6	114	91	120	105
	7	103	108	113	113
	8	120	100	110	106
	9	86	102	115	85
	10	61	96	122	113
II	1	104	95	116	105
	2	86	126	112	104
	3	98	102	107	140
	4	98	111	93	132
	5	99	72	103	114
	6	106	123	125	116
	7	89	90	117	103
	8	100	110	119	134
	9	105	108	94	104
	10	88	117	110	99
III	1	103	126	123	132
	2	112	87	152	111
	3	113	120	125	141
	4	104	77	138	122
	5	112	113	136	141
	6	95	121	119	140
	7	120	93	97	139
	8	106	120	133	123
	9	80	114	101	112
	10	101	115	103	125
Mean:		100.8	104.2	117.3	117.5
Std. Dev.:		12.5	14.0	13.4	14.9

Table A-3. Heights (mm) of Cucumber Plants on Day 14, Grown In Soil Amended With Graphite Flakes (Second Study).

Replicate No.	Plant No.	Plant Heights (mm)			
		0%	0.50%	0.10%	0.50%
I	1	96	105	102	96
	2	85	96	84	110
	3	94	112	84	107
	4	95	97	86	89
	5	99	101	103	103
	6	96	111	98	94
	7	87	98	99	97
	8	93	97	91	95
	9	91	111	89	87
	10	83	90	102	83
II	1	99	77	83	87
	2	117	102	101	99
	3	108	82	112	119
	4	116	94	92	124
	5	104	86	111	122
	6	98	107	114	113
	7	103	92	112	108
	8	113	100	111	98
	9	92	96	89	109
	10	91	89	91	104
III	1	93	102	83	83
	2	93	114	77	92
	3	97	79	107	105
	4	95	109	108	99
	5	112	109	107	97
	6	93	114	97	97
	7	94	80	92	99
	8	105	96	98	102
	9	95	96	108	0
	10	86	108	80	0
Mean:		97.4	98.3	97.0	93.9
Std. Dev.:		8.8	10.7	10.9	27.6

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APPENDIX B
STATISTICAL DATA
ANALYSIS OF VARIANCE (ANOVA) OF HEIGHTS OF CORN AND CUCUMBER PLANTS GROWN IN SOIL AMENDED WITH GRAPHITE FLAKES

Table B-1. Analysis of Variance, Two-Way, Single Observation: Plant Height (mm) of Corn (10 Plants/Block), Crown in Soil Amended with Graphite Flakes.

Parameter	Control 0%	0.05%	0.10%	0.50%
N:	30.0	30.0	30.0	30.0
Mean:	346.8	373.9	361.7	351.5
Std. Dev.	69.2	59.8	78.2	84.1

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Value
Total:	638017	119		
Treatments:	12998	3	4332.667	0.91
Blocks:	210976	29	7275.035	1.53
Within:	414043	87	4759.115	

F (95%): 2.76 F (99%): 4.13

Differences Between Treatments: Not Significant at $p < 0.05$

F (95%): 1.89 F (99%): 2.47

Differences Between Blocks: Not Significant at $p < 0.05$

Table B-2. Analysis of Variance, Two-Way, Single Observation: Plant Height (mm) of Cucumber (10 Plants/Block), Grown in Soil Amended with Graphite Flakes.

Parameter	Control 0%	0.05%	0.10%	0.50%
N:	30.0	30.0	30.0	30.0
Mean:	100.8	104.2	117.3	117.5
Std. Dev.	12.5	14.0	13.4	14.9

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Value
Total:	28715.49	119		
Treatments:	6864.625	3	2288.208	13.61
Blocks:	7228	29	249.2414	1.48
Within:	14622.87	87	168.07896	

F (95%): 2.76 F (99%): 4.13

Differences Between Treatments: Significant at $p < 0.01$

F (95%): 1.89 F (99%): 2.47

Differences Between Blocks: Not Significant at $p < 0.05$

Table B-3. Analysis of Variance, Two-Way, Single Observation: Plant Height (mm) of Cucumber (10 Plants/Block), Grown in Soil Amended with Graphite Flakes (Second Study).

Parameter	Control 0%	0.05%	0.10%	0.50%
N:	30.0	30.0	30.0	30.0
Mean:	97.4	98.3	97.0	93.9
Std. Dev.	8.8	10.7	10.9	27.6

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Value
Total:	31415.99	119		
Treatments:	329.125	3	109.708	0.44
Blocks:	9358.5	29	322.7069	1.29
Within:	21728.37	87	249.7513	

F (95%): 2.76 F (99%): 4.13

Differences Between Treatments: Not Significant at $p < 0.05$

F (95%): 1.89 F (99%): 2.47

Differences Between Blocks: Not Significant at $p < 0.05$

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APPENDIX C
PLANT DATA
DRY WEIGHTS (g) OF CORN AND CUCUMBER PLANTS
GROWN IN SOIL AMENDED WITH GRAPHITE FLAKES

Table C-1. Dry Weights (g) of Corn Plants on Day 14, Grown In Soil Amended With Graphite Flakes.

Replicate No.	Dry Weights			
	0%	0.05%	0.10%	0.50%
I	0.9159*	1.0104	0.9173	0.7199
II	0.9408	0.9704	0.6800	1.1292
III	0.7577	0.9516	1.0242	0.8281
Mean:	0.8715	0.9775	0.8738	0.8924
Std. Dev.:	0.0993	0.0300	0.1761	0.2121

* Weight in grams of all 10 plants/treatment.

Table C-2. Dry Weights (g) of Cucumber Plants on Day 14, Grown In Soil Amended With Graphite Flakes.

Replicate No.	Dry Weights (g)			
	0%	0.05%	0.10%	0.50%
I	0.6086*	0.5639	0.6559	0.6797
II	0.4872	0.5359	0.5591	0.5703
III	0.5861	0.6431	1.6411	0.6482
Mean:	0.5606	0.5810	0.6187	0.6327
Std. Dev.:	0.0646	0.0556	0.0521	0.0563

* Weight in grams of all 10 plants/treatment.

Table C-3. Dry Weights (g) of Cucumber Plants on Day 14, Grown In Soil Amended With Graphite Flakes (Second Study).

Replicate No.	Dry Weights (g)			
	0%	0.05%	0.10%	0.50%
I	0.6012*	0.6998	0.6305	0.6525
II	0.7313	0.6847	0.7075	0.7598
III	0.7037	0.7524	0.6706	0.5852
Mean:	0.6787	0.7123	0.6695	0.6658
Std. Dev.:	0.0685	0.0355	0.0385	0.0881

* Weight in grams of all 10 plants/treatment.

APPENDIX D
STATISTICAL DATA
NEWMAN-KEULS ANALYSIS OF TREATMENT
OF CUCUMBER PLANT HEIGHTS

Table D-1. Newman-Keuls Analysis of All Treatments, Pairwise, and Ranked From Low to High: Cucumber Plant Heights (mm), Grown in Soil Amended with Graphite Flakes.

Treatment (%)	0%	0.05%	0.10%	0.50%
		q values		
0		1.357	6.585*	6.691*
0.05			5.228*	5.334*
0.10				0.106
0.50				
q (95%)		2.80	3.36	3.69
q (99%)		3.70	4.21	4.51

*Significant at $p < 0.01$

QUALITY ASSURANCE

This study was examined to determine compliance with applicable SOP's governing the testing. The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

Phase inspected	Date	Date reported
Data and Final report	12 June 1990	12 June 1990

To the best of my knowledge, the methods described were the methods followed during the study. The report was determined to be an accurate reflection of the raw data obtained.

Kenneth P Cameron 13 June 90

KENNETH P. CAMERON
Quality Assurance Specialist